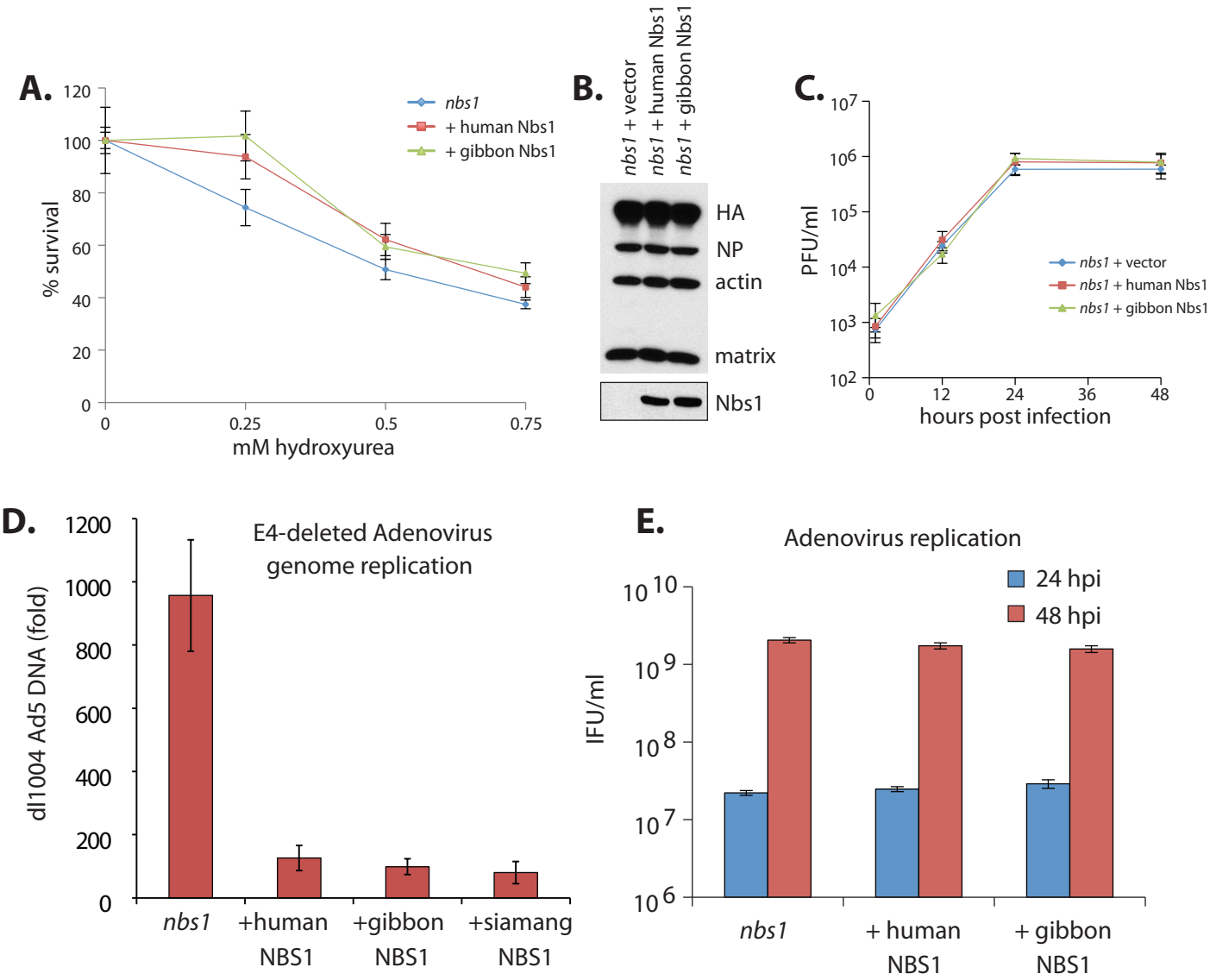
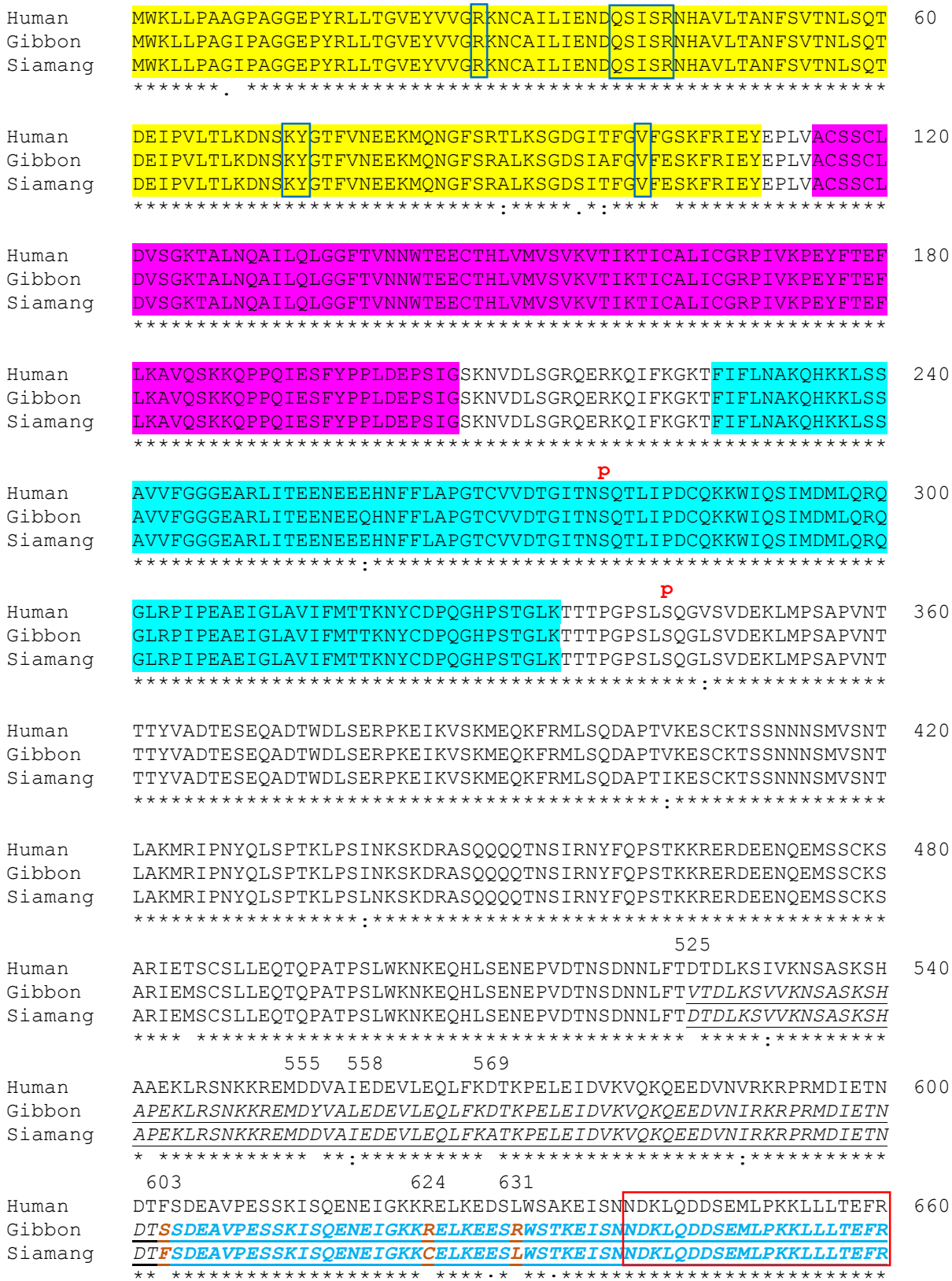


Figure S1, related to Figure 2. Primate divergence in Nbs1 does not affect sensitiveity to genotyoxins or other viruses. **(A)** nbs1 cells stably expressing empty vector, human, or gibbon Nbs1 were exposed to increasing doses of hydroxyurea. Colony formation was assessed after 7-10 days. Percent survival was calculated by normalizing the number of colonies to untreated controls. Experiments were performed in triplicate and error bars represent standard deviations from the mean. **(B, C)** nbs1 cells complemented with the indicated constructs were infected with the A/Udorn/H3N2 influenza A virus at an MOI of 2. Cells were washed with PBS and then incubated in infection media (DMEM supplemented with Pen/Strep, L-Glut, and 1% BSA) for one hour at 37°C. Cells were washed once more in PBS and incubated in influenza growth media (DMEM supplemented with Pen/Strep and L-Glut; multiple-cycle samples also included 0.5 ug/ml N-acetylated trypsin). **(B)** Cell lysates were harvested at 12 hours post infection using RIPA buffer supplemented with complete protease inhibitor (Roche) and PMSF (Invitrogen). Whole cell lysates were subjected to western blotting and influenza proteins were visualized using a goat polyclonal anti-Udorn antibody (PMID: 19008398) that recognizes hemagluttinin (HA), nucleoprotein (NP), and matrix (M). Nbs1 and actin expression levels were also determined. **(C)** Supernatants were collected at 1, 12, 24, and 48 hours post infection and titrated by carrying out a plaque assay on MDCK reporter cells. Infections for plaque assays were carried out as described above, except the final incubation media contained 1.4% Avicel (Sigma-Aldrich) to induce plaque formation. The results shown are an average of three independent replicates, with error bars representing standard deviations. **(D,E)** Adenovirus replicates within the nucleus, where its double-stranded DNA genome is recognized by the MRN complex. To evade detection by MRN and promote viral replication, viral proteins expressed from the E4 region of the adenovirus genome mislocalize and degrade MRN (Stracker et al. 2002). An E4-deleted adenovirus mutant (dl1004) does not target MRN, and MRN localizes to viral DNA (Stracker et al. 2002). Infection of nbs1 cells with dl1004 results in wild-type levels of viral replication, as the MRN complex does not form in these cells (Desai-Mehta et al., 2001). However, when nbs1 cells are complemented with wild-type human Nbs1, there is a dramatic reduction in replication of the mutant virus, demonstrating that human Nbs1 restricts adenovirus replication (Lakdawala et al. 2008). **(D)** nbs1 cells complemented with an empty vector, human Nbs1, siamang Nbs1, or gibbon Nbs1 were infected with an E4-deleted adenovirus mutant dl1004 at MOI 20 for two hours in DMEM supplemented with 2% FBS and 1% Pen/Strep. After adsorption, infection media was replaced with DMEM containing 10% FBS and 1% Pen/Strep. Infected cells were harvested with Trypsin at 4 and 30 hours post infection, and DNA was isolated using the PureLink Genomic DNA kit (Thermo Scientific). Quantitative PCR was performed using primers specific for the viral DBP gene and cellular tubulin, as described previously (Lakdawala et al. 2008). Values for DBP were normalized first to tubulin and then to the 4 hour time point to control for any variation in input virus. Fold change over input of adenovirus DNA is shown above. Results are an average of three biological replicates. Error bars represent standard deviation. **(E)** nbs1 cells complemented with empty vector, human Nbs1, or gibbon Nbs1 were infected with wild-type adenovirus type 5 at an MOI of 5 in triplicate. After 24 and 48 hours post infection, supernatants were collected and titrated on HEK-293 cells using the Adeno-X Rapid Titer Kit (ClonTech) to quantify virus production. The results are expressed as an average of three replicates, and error bars represent standard deviations.

Figure S2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Gibbon LKAVQSKKQPPQIESFYPPLDEPSIGSKNVDLSGRQERKQIFKGKTFIFLNAKQHKKLSS
Siamang LKAVQSKKQPPQIESFYPPLDEPSIGSKNVDLSGRQERKQIFKGKTFIFLNAKQHKKLSS
p
Human AVVFGGGEARLITEENEEEHNFFLAPGTCVVDTGITNSQTLIPDCQKKWIQSIMDMLQRQ 300
Gibbon AVVFGGGEARLITEENEEQHNFFLAPGTCVVDTGITNSQTLIPDCQKKWIQSIMDMLQRQ
Siamang AVVFGGGEARLITEENEEEHNFFLAPGTCVVDTGITNSQTLIPDCQKKWIQSIMDMLQRQ
p
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Gibbon GLRPIPEAEIGLAVIFMTTKNYCDPQGHPSTGLKTTTPGPSLSQGLSVDEKLMPSAPVNT
Siamang GLRPIPEAEIGLAVIFMTTKNYCDPQGHPSTGLKTTTPGPSLSQGLSVDEKLMPSAPVNT
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Gibbon TTYVADTESEQADTWDLSERPKEIKVSKMEQKFRMLSQDAPTVKESCKTSSNNNSMVSNT
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Siamang DTFSDEAVPESSKISQENEIGKKCELKEESLWSTKEISNNDKLQDDSEMLPKKLLLTEFR

Figure S2, related to Figures 3 and 7. Protein sequence alignment of the human, gibbon, and siamang Nbs1. A protein sequence alignment was generated using ClustalX. The FHA, BRCT1, and BRCT2 domains are shown in yellow, purple, and blue, respectively (Lloyd et al., 2009; Williams et al., 2009). Sites of phosphorylation are indicated in red (Lloyd et al., 2009). Blue open boxes highlight residues important for binding CtIP (Williams et al., 2009), red boxes are residues that mediate interaction with Mre11 (Schiller et al., 2012) and grey boxes are amino acids involved in ATM binding (Falck et al., 2005). Swapped regions in the -4 and -8 chimeras are underlined in bold italics. The smaller region (-4) containing amino acids 603, 624, 631, and 673 is shown in blue text.

Supplemental Experimental Procedures

Generation of stable cell lines expressing primate NBS1. The human *NBS1* ORF template was a kind gift from Tanya Paull. Clones of primate *NBS1* were created as follows. Total RNA was extracted from B lymphocytes or fibroblasts of *Pongo abelii* (orangutan), *Macaca mulatta* (rhesus), *Nomascus leucogenys* (gibbon), and *Symphalangus syndactylus* (siamang) using the RNeasy Mini Kit (QIAGEN). cDNA was generated using the SuperScript III First-Strand Synthesis System (Invitrogen). *NBS1* open reading frames were then amplified with a C-terminal FLAG tag and cloned into the pLNCX-m1 retroviral transfer vector (Clontech). GenBank accession numbers are as follows: *Homo sapiens* (human, AAC39752), *Pongo abelii* (orangutan, NM001204362), *Macaca mulatta* (rhesus, NM001265739), *Nomascus leucogenys* (gibbon, HM486828), and *Symphalangus syndactylus* (siamang, HM486829). Retroviruses were packaged in HEK-293T cells by co-transfecting the transfer vector with the NB-MLV pCS2-mGP packaging plasmid (Yamashita and Emerman, 2004) and pC-VSV-G (provided by Hyeryun Choe). Supernatants containing retrovirus were collected 48 hours post transfection and filtered through a 0.45 μm nitrocellulose membrane. NBS1-lLB1 cells were plated at 15% confluency in a 12 well plate and transduced with varying amounts of each retrovirus and 5 μg/ml polybrene. The plates were spinoculated at 1,200 g for 90 minutes at 30˚C and incubated at 37˚C for 24 hours. The media was replaced with selection media consisting of DMEM with 10% FBS, 100 μg/ml streptomycin, 100 U/ml penicillin, and 0.8 μg/ml of G418, and maintained under selection for at least 1 month.

HSV-1 infection and plaque assays. Virus stocks were grown on Vero cells. Infections were conducted on cell monolayers at the indicated MOIs in serum free media for 1 hour at 37°C. The cells were washed with PBS and complete media containing 10% FBS and Pen/Strep was added. Supernatants from infected cells were collected at the indicated time points. For plaque assays, 10-fold serial dilutions were made and titrated on Vero cells. After 1 hour of adsorption, media containing 10% FBS and 1% human serum was added. Plaques were stained using a 0.5% crystal violet, 25% methanol solution after 2-3 days. Alternately, cells were fixed with 2% paraformaldehyde and stained with an X-gal staining solution. Where indicated, 200 μg/ml phosphonoacetic acid (PAA, Sigma) was added at the time of infection.

Immunoblotting: Expression of Nbs1 proteins was confirmed by collecting a confluent well in a 6 well plate. Supernatants were aspirated and cells were washed two times with ice-cold PBS. Cells were lysed and collected in 100 μl of RIPA buffer (150 mM NaCl, 50 mM Tris pH 8.0, 0.1% SDS, and 1% NP-40). Lysates were transferred to microfuge tubes and rotated at 4˚C for 1 hour. Lysates were cleared by centrifugation at max speed for 10 minutes. Cleared lysates were transferred to a new microfuge tube. Expression of Nbs1 proteins was detected by running 60 μg of total protein denatured in SDS loading buffer on a 4-20% Tris-glycine SDS PAGE gel. Proteins were transferred to a nitrocellulose membrane using the Mini Trans-Blot Electrophoretic Transfer Cell (BioRad) for 90 minutes at 100 V. Membranes were blocked with 5% milk in TBS-T (0.1%) for 1 hour at 4˚C with gentle rocking. Membranes were washed 3 times with TBS-T (0.1%) and incubated with a 1:5000 dilution of the Nbs1 specific antibody Y112 (Genetex) or the FLAG C2 antibody (Syd Labs), for 1 hour at room temperature or overnight at 4˚C with gentle rocking. Membranes were washed 3 times with TBS-T (0.1%) and finally incubated with a 1:5000 dilution of the appropriate secondary antibody. Membranes were visualized using Amersham ECL Prime Western Blotting Detection Reagent (GE Life Sciences). Expression of all other human endogenous and HSV-1 proteins were analyzed in the same manner as described above.

Co-IP of MRN components: For the co-IP of MRN components, a confluent 100 mm dish of NBS-1LBI (herein, *nbs1*) cells expressing empty vector, human, or gibbon Nbs1 was lysed in 500 µl of ice-cold lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.5% NP-40) and rotated for at least 1 hour at 4°C. The lysates were cleared and a 15 µl aliquot was saved as the input sample. The remaining samples were incubated with 10 µl of anti-DYKDDDDK conjugated magnetic beads (Syd labs) at 4°C for at least 2 hours. The beads were washed 3 times with lysis buffer and 3 times with Buffer A (25 mM Tris pH 8, 100 mM NaCl, 10% v/v glycerol, 1 mM DTT). Bound proteins were eluted with a DYKDDDDK peptide (Syd Labs). SDS loading buffer was added to the samples and boiled for 10 minutes. The samples were separated via gel electrophoresis on a polyacrylamide gel and transferred to a 0.45 µm pore size nitrocellulose membrane. The membrane was blocked in 5% milk in TBS-Tween (0.1%) and immunoblotting was carried out using a primary antibody in TBS-T (0.1%) (1:5000 Nbs1 Y112, 1:1000 Mre11 12D7, or 1:1000 Rad50) and 1:5000 diluted rabbit or mouse HRP-conjugated secondary antibody in TBS-T (0.1%) (Thermo Scientific). Amersham ECL Prime Western Blotting Detection Reagent (GE Life Sciences) was used for visualization.

Co-IP of ICP0 and Nbs1: For the co-IP of ICP0 and Nbs1, 6 well plates containing 8 x 10^5/well HEK-293Ts were transfected with the indicated plasmids using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. At 48 hours post transfection, cells were harvested in 500 µl of ice-cold co-IP buffer with protease inhibitors (50 mM Tris-HCl pH7.4, 150 mM NaCl, 0.1% Triton X-100, 50 mM NaF, 1 mM sodium vanadate) and subjected to mild sonication. The lysates were cleared and 10% of the sample was saved as the input sample. The remaining samples were incubated with 5 µg of anti-GFP antibody per sample (Abcam) for at least 2 hours at 4°C with constant rotation. 20 µl of Dynabeads Protein G (Novex) were then added to the samples and rotated at 4°C for at least 1 hour. The beads were washed four times with ice-cold co-IP buffer and resuspended in SDS loading buffer. Immunoblotting was performed as described in the previous section using a 1:1000 dilution of the ICP0 (Santa Cruz) and a 1:2000 dilution of the FLAG M2 (Sigma) antibodies. For endogenous co-IPs, HFF (3 x 10^6 in 100-mm dish) or NBS-1LBI (6 x 10^6 in 100-mm dish) cells were mock infected or infected with HSV-1 at an MOI of 3. Cells were harvested at indicated time point and sonicated in 500 ul of co-IP buffer. The clarified cell lysates were incubated with 5 ug of anti-ICP0 antibody (Santa Cruz) per sample for 2 hours at 4'C. 30 ul of Dynabeads Protein G were incubated for 1 hour. The beads were then washed with co-IP buffer and resuspended in SDS loading buffer. Immunoblotting was performed with anti-ICP0 (1:1000) mouse monoclonal and anti-Nbs1 (Novus, 1:1000) rabbit polyclonal antibodies.

Viral DNA and mRNA detection: For viral DNA quantification, DNA was extracted from cells using the PureLink Genomic DNA Extraction Mini Kit (Invitrogen) and quantified by amplifying the HSV-1 *ICP27* gene. These values were normalized to the endogenous control gene *RPLP0*. For HSV-1 mRNA quantification, total RNA was isolated (RNeasy Mini Kit, Qiagen) and was reverse transcribed using a high-capacity RNA-to-cDNA kit (Applied Biosystems). Products were amplified using the Power SYBR Green PCR Master Mix (Applied Biosystems) on the ViiA7 Real-Time PCR System (Applied Biosystems) using the following primers: ICP27 (HSV-1) F: gcatccttcgtgtttgtcatt, R: gcatcttctctccgaccccg; RPLP0 control F: ctggaagtccaactacttcc, R: tgctgcatctgcttggagcc.

Immunofluorescence: Cells were seeded at a density of 8 x 10^4 per well in 24 well plates containing glass coverslips coated with poly-L lysine. The next day, cells were infected with HSV-1 at an MOI of 10. At 2 hour post-infection, cells were washed with PBS and fixed with 4% paraformaldehyde for 15 minutes and permeabilized with 0.5% Triton X-100 in PBS for 10 min.

Coverslips were incubated for 1 hour with the ICP4 (1:200) or FLAG rabbit F7425 (1:2000) antibody in PBS at room temperature and then incubated with secondary antibodies Alexa Fluor 488 goat anti-mouse IgG (1:200) or Alexa Fluor 568 goat anti-rabbit IgG (1:200) (Life Technologies). Nuclei were visualized by staining with 1 µg/ml DAPI (Thermo Fisher, D1306).

Structural Disorder in Virus Interacting Proteins: The sets of mammalian proteins that do and do not interact with viral DNA, RNA, or protein are from (Enard et al., 2016). They include 1,237 virus-interacting mammalian proteins and 8,356 mammalian proteins that do not interact with viruses. Each protein in this dataset has a fraction of their sequence predicted to be disordered that ranges from zero to one. The distribution of the disorder in virus-interacting proteins was compared to the same distribution in proteins that do not interact with viruses. To test if the degree of structural disorder is different between the two groups of proteins, we used simple random permutations. We first measured the average fraction disordered in the 1,237 virus-interacting proteins, and then compared it with the average from 1,237 proteins randomly sampled out of the 8,356 proteins that do not interact with viruses. By repeating this random sampling 100,000 times, each time comparing the random average with the observed average in the virus-interacting proteins, we calculated an observed empirical p-value. The low empirical p-value obtained ($p = 0$) is indicative of a strongly significant difference in disorder between the two groups of proteins, those that interact with and those that do not interact with viruses.

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